

USDA APHIS Veterinary Services

Chronic Wasting Disease Program – Sample Collection Guidance

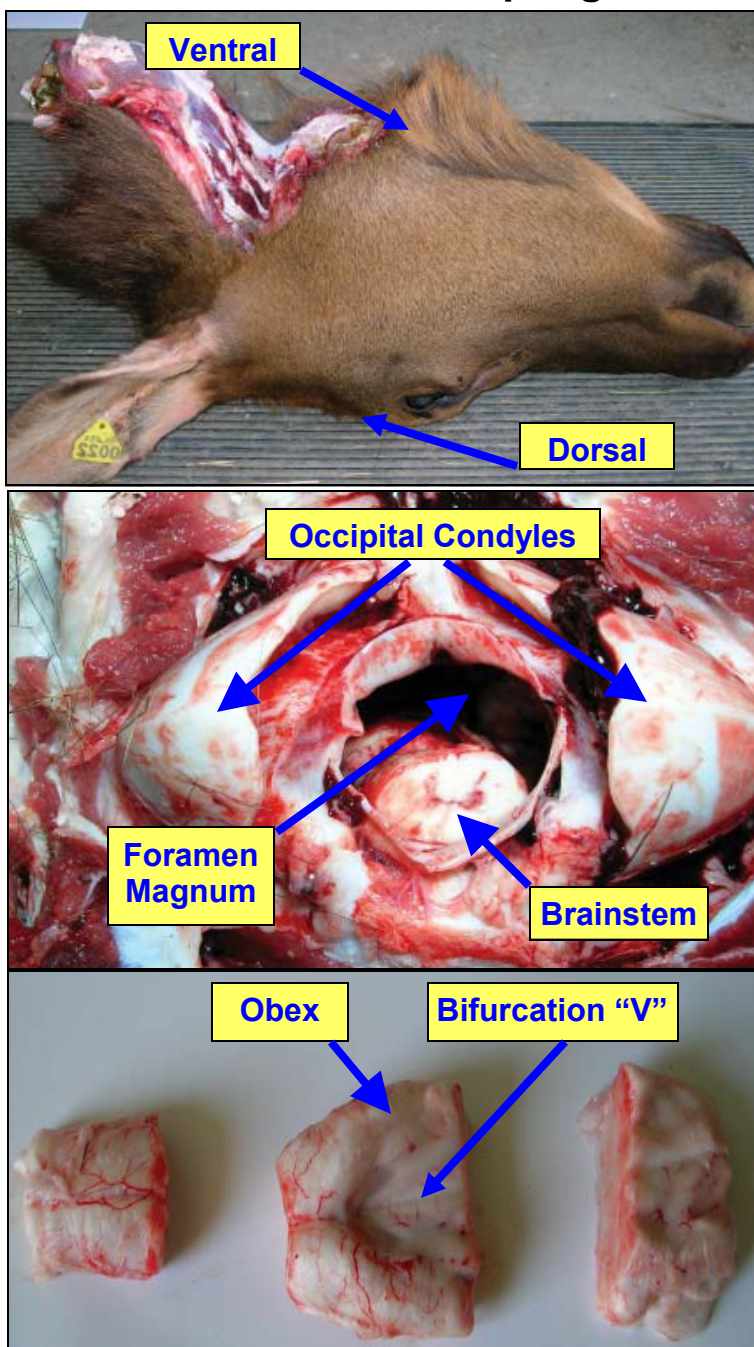
Procedure for Removal of Obex & Retropharyngeal Lymph Nodes (RPLN)

Chronic Wasting Disease (CWD) has not been shown to infect humans, but other cervid diseases, such as rabies, can be zoonotic diseases. Therefore, personal protective equipment (PPE) should be worn when handling animal tissues. If the animal shows signs consistent with CWD or other neurologic diseases, please contact your APHIS Area Office and/or State Animal Health agency for further guidance.

Equipment Needed: PPE with gloves, Extraction Spoon, Scapel, Forceps, Scissors, Knife, Formalin Jars or Conical Tubes, Whirl-pak® / Zip Lock Bags, Permanent Marker, Labels, Ice Packs, Coolers, Disinfectant.

PLEASE NOTE: Obex & medial RPLN are required tissues to test for APHIS CWD Herd Certification Program (HCP). Immunohistochemistry (IHC) is the official HCP test method.

Sampling Procedure for Obex:



1. Incise the head of the animal at the atlanto-occipital joint (between skull and first vertebra). Cut behind the back of the ears and extend the cut around and through the front of the larynx. Sever the brainstem as far to the posterior as possible during the removal process.
2. Position the head upside down (ventral side up). Locate the occipital condyles and foramen magnum (FM). Locate the brainstem inside the FM. Trim the dura mater around the brainstem and cut the attached cranial nerve trunks.
3. Gently lift the brainstem with forceps and insert the spoon into the **dorsal** aspect of the FM between the brainstem and **dorsal** calvarium.
4. Advance the spoon 2-3 inches rostrally until it contacts bone to sever the cerebellum.
5. Re-position the spoon in the **ventral** aspect of FM between the brainstem and the **ventral** calvarium. Advance the spoon until it contacts bone and transversely sever the brainstem.
6. Remove the brainstem using the spoon and forceps. Examine to ensure the proper obex sample (Bifurcation or "V") is preserved.
7. Further trim the brainstem section by making a transverse cut 3/4 inch in front of the "V" shape bifurcation and an equal distance behind the bifurcation for good fixation.

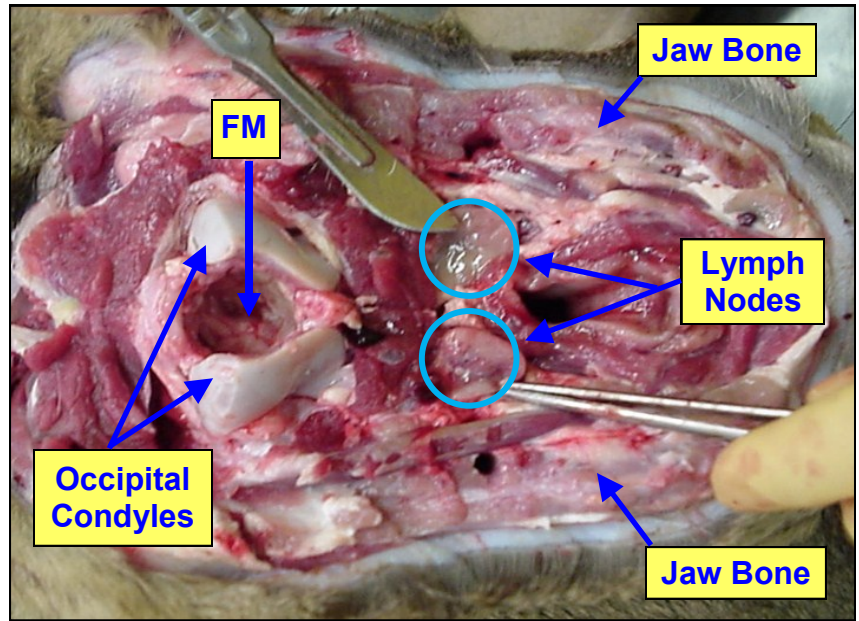
For IHC testing – place the trimmed obex and brainstem pieces in 10% buffered formalin jar (10:1 ratio of formalin to tissue sample).

For ELISA testing – place the fresh obex sample and trimmed pieces in a conical tube (NO Formalin).

Please see Sample Submission Procedure section for further details.

Sampling Procedure for Medial Retropharyngeal Lymph Node:

1. With the head positioned upside down, locate the esophagus and trachea above the foramen magnum (FM).
2. Lift the trachea and dissect muscles forward of the foramen magnum (rostrally). Locate the left and right medial Retropharyngeal Lymph Nodes (RPLN) half-way between each corner of the jaw bone and the FM, caudal to the nasopharynx, and deep to the salivary gland. LN consistency is much firmer and rounder than the surrounding tissue.
3. Remove each left and right medial RPLN and longitudinally incise each LN to confirm lymphoid tissue.



- **For IHC testing** – place the medial RPLNs in the same formalin jar with the obex.
- **For ELISA testing** – place the fresh medial RPLNs in labeled whirl-pak bags.

Photo courtesy: MD DNR

Animal/Sample Identification and Chain of Custody Validation

Photo courtesy: APHIS



Remove a **piece** of the ear (or a piece of hide if a trophy animal) attached to the official ID device and place in a whirl-pak or other sealable bag. Label whirl-pak bag and specimen containers with sample number and animal information (producer name, official ID number, species, date, etc). Place bagged ear/hide in freezer until ready to ship with the CWD samples.

Use an ice pack when shipping the ear/hide specimens. If there is no official ID in the ear, the CWD sample collector must affix one in the ear and record the information on the samples and submission forms. For a trophy animal, place official ID in the hide and submit the ID with attached piece of hide in lieu of a piece of ear. Some labs may accept ID samples in formalin. Verify with the receiving lab.

Biosecurity and Decontamination

Dedicated or disposable instruments, work area covers, and PPE are recommended for sampling. Contaminated surfaces and instruments may be soaked in undiluted (5%) bleach for 1 hour to reduce any prion contamination. Change clothes and wash thoroughly before leaving the premises and/or before coming into contact with any live cervids.

Sample Submission Procedure

1. For **Category B (UN3373)** packaging & shipping details see the following website, or contact the receiving lab or NVSL: www.aphis.usda.gov/animal_health/lab_info_services/packaging_labeling.shtml
2. Label sample containers / bags with sample number, producer name, official ID number, species, date, etc.
 - **IHC testing:** Ship samples in formalin jars.
 - **ELISA testing:** Keep samples chilled on icepacks. Ship using overnight delivery.
3. Complete **VS 10-4 Form**. Be sure to indicate if the animal was an exposed animal and any clinical signs. List any signs in the Additional Data section of the VS 10-4 form.
4. Ship to a VS approved lab or to NVSL with proper authorization (ex. AVIC approval).

